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Hartmann, Nanna Isabella Bloch; Buendia, Inmaculada M.; Bak, Jimmy; Baun, Anders

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Degradability of aged aquatic suspensions of C_{60} nanoparticles

Nanna B. Hartmann[a,*], Inmaculada M. Buendia[a], Anders Baun[a]

[a]Department of Environmental Engineering, Technical University of Denmark

Building 113, Kgs. Lyngby, Denmark

* Corresponding author. Postal address: Department of Environmental Engineering, Technical University of Denmark, Miljoevej, Building 113, DK-2800 Kgs. Lyngby, Denmark. E-mail: nibh@env.dtu.dk. Tel.: (+45) 4525 2164. Fax.: (+45) 4593 2850.

ABSTRACT

In this study, aged aqueous suspensions of C_{60} (nC_{60}) were investigated in the respirometric OECD test for ready biodegradability. Two suspensions of nC_{60} were prepared by stirring and aged under indirect exposure to sunlight for 36 months. The suspensions consisted of particle aggregates with a mean size around 150 nm, but also contained smaller aggregates. Samples of the nC_{60} suspensions (20 mg/l) were inoculated with activated sludge (30 mgTSS/L) and incubated in a mineral medium under aerobic conditions. Since no mineralization of nC_{60} was observed after 28 days of incubation, 5 mg/l sodium acetate was added to the media. After additional 20 days, no mineralization of nC_{60} was observed. However, within a few days sodium acetate was completely mineralized, showing that the biomass was not inhibited by the presence of nC_{60}. Based on these results, aged nC_{60} can be classified as not ready biodegradable according to the OECD test procedure.

Capsule:

Aged suspensions of nC_{60} were found not to be ready biodegradable according to the OECD test procedure. The biomass was not inhibited by the presence of nC_{60}.

INTRODUCTION

While studies of the potential human and environmental effects of C_{60} and its derivatives are emerging in the scientific literature, the environmental fate of C_{60} is still largely unknown (Hou & Jafvert, 2008; Baun et al., 2009). C_{60} has many and diverse potential applications, but very low water solubility hampers the use of C_{60} especially in biological applications. However, stable aqueous suspensions of C_{60} (referred to as nC_{60}) can be prepared with or without the addition of solvents. Suspensions of nC60 may be prepared using different techniques such as:solvent exchange method (Deguchi et al. 2001; Fortner et al. 2005), ultrasonication (Lee et al., 2009, Henry et al., 2007) and prolonged stirring in water (Chen and Elimelech, 2009. Oberdörster et al., 2006).

In its pristine form, C_{60} is considered to be recalcitrant to microbial degradation due to its stable closed-cage structure. However, when transformed into more water soluble forms by abiotic processes (Hou & Jafvert 2008; Lee et al., 2009), the carbon cage structure can become more susceptible to biological degradation. For instance, a recent study has shown that fullerol ($C_{60}(OH)_{19\text{-}27}$) can be oxidized by white rot basidiomycete fungi to CO_2 (Schreiner et al., 2009) and uptake of the ^{13}C from labelled fullerols into biomass was observed.

Although the abiotic transformation processes of C_{60} in aqueous suspensions is an area of great importance in environmental chemistry, data on the total mineralization of carbon-containing substances are needed for hazard identification and labelling, e.g. in the global harmonized system of Classification and Labelling of Chemicals (EU, 2008)., The tiered

OECD test strategy (OECD, 1992) is the internationally accepted procedure to generate data on mineralization of chemicals in water. However, to the best of our knowledge, no experiments on biodegradability of nC_{60} in OECD tests exists at present. The aim of this study is therefore to provide the first data on the biodegradability of aged nC_{60} in a test following one of the OECD guidelines for ready biodegradability, i.e. the first level in the OECD test strategy for biodegradability.

MATERIALS AND METHODS

Preparation of aqueous C_{60} suspensions

Two aqueous suspensions of nC_{60} were prepared by adding 100 mg C_{60} (purity 98%, Sigma) to 1 L of MilliQ water followed by constant stirring in the laboratory and exposed indirectly to natural sunlight for 36 months (N55.79, E12.53). Prior to the degradability experiments, the suspensions were left to sediment for 24 hours and the two supernatants (termed A & B) were decanted. The stability of the supernatants was investigated by following the change in absorbance (Varian Cary 50 Bio UV-Vis) at 350 nm over a period of 72 hours and was found to be stable after initial settling of larger aggregates (See supporting info).

Test for ready biodegradability

The test for ready biodegradability was carried out according to the OECD 301F standard procedure (OECD, 1992) using the OxiTop® instrumentation. The two suspensions (A and B) of nC_{60} were added to separate test bottles in a concentration of approximately 20 mg/L in the OECD mineral medium (OECD, 1992). These were

inoculated with activated sludge (30 mgTSS/L) and incubated for 28 days in the dark under aerobic conditions with constant stirring for 28 days. The activated sludge was sampled at the Lundtofte wastewater treatment plant (Kgs. Lyngby, Denmark). Determination of total suspended solids (TSS) in the activated sludge was performed according to Standard Methods (Standard Methods, 1998). Two bottles without substrate were prepared in order to assess the background O_2 consumption of the biomass. Additionally, two bottles with 10 mgC/L sodium acetate (NaAc) were included in the experiment as positive controls for ready biodegradability. After 28 days of incubation, 5 mgC/L NaAc were added to the bottles containing C_{60}, as well as to the reference bottles, leaving them for incubation for 20 days more.

Chemical analyses & characterisation

Concentrations of nC_{60} (pristine C_{60} and transformation products in suspension) in the two suspensions, were estimated from several different measurements. COD measurements were performed according to Standard Methods using $K_2Cr_2O_7$ as oxidizing agent (Standard Methods, 1998). Oxidation of C_{60} by $K_2Cr_2O_7$ has previously been observed by Ko & Baek (2002) and Chibante et al (1994). The applicability of the method was investigated by applying the technique to samples of pure C_{60} powder and to aged nC_{60} suspensions. These tests showed that complete oxidation did not occur for C_{60} in powder form (≈ 5-15% recovery). However, for nC_{60} suspensions, no precipitate was seen and 75-106% recovery of the added C_{60} was found (data not shown). Total-carbon concentrations (TOC) were measured using Shimadzu TOC-Vwp equipment with ASI-V auto sampler. The concentration of C_{60} in the solutions was also determined as described

by Fortner et al. (2005) and Baun et al. (2008). Briefly, the method includes a two-step destabilization-extraction procedure using $Mg(ClO_4)_2$ for oxidation and toluene for extracting. Absorbance was measured at a wavelength of 336 nm and nC_{60} concentrations calculated based on a standard curve of C_{60} dissolved in toluene.

Additional characterization of the nC_{60} suspensions were done by measuring the absorbance spectra at 350 nm without any sample preparation (Supporting Info). Also the nC_{60} suspensions were dried on square mesh copper grids with holey carbon support films and characterised by TEM (Tecnai T20 G2 TEM from FEI (Oregon, USA) operated at 200 kV). Size distributions were investigated by means of Nanoparticle Tracking Analysis (Nanosight LM10, NTA 2.1. software).

RESULTS & DISCUSSION

Ready biodegradability test

Figure 1 shows the biological oxygen demand (BOD) as percentage of the theoretical oxygen demand (ThOD) for the bottles containing C_{60} (A & B) and NaAc with the BOD of blank experiments substracted. No lag-phase was observed for NaAc mineralization, reaching more than 60% ThOD after 5 days of incubation (Figure 1). The samples containing nC_{60} showed no sign of mineralization during the 28-days period. 5 mg/l sodium acetate was then added and after additional 20 days, no mineralization of nC60 was observed. However, within a few days sodium acetate was completely mineralized, showing that the biomass was not inhibited by the presence of nC_{60}.

Chemical analysis and characterisation of nC_{60}

By comparing initial and final C_{60} concentrations (Table 1) it is evident that the overall concentration of carbon is not considerably reduced during the test period. It should be noted that final COD values comprise oxygen demand from both nC_{60}, and biomass. Therefore, the initial biomass (calculated as 30 mgTSS/L*1.065mgCOD/mgTSS = 32mgCOD/L (Henze et al., 2008)), was subtracted from final COD. Due to microbial growth as a result of the mineraliztion of of NaAc, this may underestimate the actual biomass contribution to COD at the end of the test, which can explain the higher COD compared to test start.

Comparing nC_{60} concentrations, estimated from COD, with results from TOC analysis ($C_{initial}$: 3.1mgC/L, C_{final}: 4.3 mgC/L), TOC measurements are much lower. This might be explained by incomplete oxidation of the samples and/or sedimentation of the nC_{60} during TOC analysis. However, the results give a qualitative verification of the fact that nC_{60} concentrations do not decrease during the test.

The presence of aggregates of nC_{60} in the two solutions is illustrated in the TEM images shown in Figure 2. From inspection of TEM images (Figure 2) many aggregates were found to be <100nm in size but also larger aggregates were found. By particle tracking analysis the mean aggregate sizes were found to be 156 nm (SD = 54 nm) for suspension A and 139 nm (SD=49 nm) for suspension B.

Recent studies have demonstrated that nC_{60} clusters can be transformed into smaller clusters or water soluble products in the presence of either UV light or sunlight. Pristine nC_{60} has been found to transform into products with mono- and dioxygenated functionalities and disaggregate into smaller clusters as a result of UV radiation (Lee et al., 2009). Furthermore, nC_{60}, prepared through an intermediate dissolution in either THF or toluene, were found to undergo photochemical transformation in sunlight (Hou & Jafvert 2008). During the irradiation period, the concentration of $nC_{60,}$ as well as cluster size, decreased and the suspensions changed colour from brown/orange to almost transparent. nC_{60} suspensions is known to show characteristic molecular absorption peaks at 260 and 350 nm (Lee et al 2009) and absorbance scans of suspensions used in this study showed such peaks, which confirms the presence of C_{60} (Supporting Info). By

visual inspection, C_{60} was not extracted into a toluene phase after oxidation with $Mg(ClO_4)_2$, and was instead present in the water phase and on the interface between the two phases. The absence of C_{60} in the toluene phase was confirmed by UV-VIS measurements (Supporting Info). These results show that the nC_{60} is no longer present as pristine particles, and that this method cannot be used to quantify total concentrations nC_{60} transformation products.

CONCLUSION

While identifying and investigating abiotic transformation processes of C_{60} in aqueous suspensions is of great importance to understand its environmental fate, data on total mineralization will be of key importance for hazard identification and labelling. Aged suspensions of nC_{60} were found not to be not ready biodegradable when tested according to the 301F OECD test procedure. Addition of NaAc did not stimulate degradation of nC_{60}. The lack of degradation was not caused by an inhibitory effect of nC_{60} on microorganisms. The low recovery of organic carbon in the TOC analysis would present a problem if the OECD 301A test (DOC Die-Away test) were to be performed with nC_{60}.

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184 REFERENCES

185

186 Baun, A., Sørensen, S.N., Rasmussen, R.F., Hartmann, N.B., Koch, C.B., 2008. Toxicity
187 and bioaccumulation of xenobiotic organic compounds in the presence of aqueous
188 suspensions of aggregates of nano-C60. Aquatic Toxicology 86, 379–387.

189

190 Baun, A., Hartmann, N.B., Grieger, K.D., Hansen, S.F., 2009. Setting the limits for
191 engineered nanoparticles in European surface waters – are current approaches
192 appropriate? Journal of Environmental Monitoring 11, 1774–1781.

193

194 Chen, K.L., Elimelech, M., 2009. Relating Colloidal Stability of
195 Fullerene (C60) Nanoparticles to Nanoparticle Charge and Electrokinetic Properties.
196 Environmental Science and Technology 43, 7270–7276.

197

198 Chibante, L.P.F, Wolbach, W.S., Heymann, D., 1994. Determination of nanogram
199 amounts of C60 by high pressure liquid chromatography. Abstracts of the 25th Lunar
200 and Planetary Science Conference, held in Houston, TX, 14-18 March 1994.

201

202 Deguchi, S., Alargova, R.G., Tsujii, K., 2001.Stable Dispersions of Fullerenes, C60
203 and C70, in Water. Preparation and Characterization. Langmuir 17, 6013-6017.

204

205 EU, 2008. Regulation (EC) No 1272/2008 of the European Parliament and of The
206 Council of 16 December 2008 on classification, labeling and packaging of substances and

mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. Official Journal of the European Union, L353, 1-1355.

Fortner, J.D., Lyon, D.Y., Sayes, C.M., Boyd, A.M.m Falkner, J.C., Hotze, E.M., Alemany, L.B., Tao, Y.J., Guo, W., Ausman, K.D., Colvin, V.L., Hughes, J.B., 2005. C60 in Water: Nanocrystal Formation and Microbial Response. Environmental Science and Technology 39, 4307-4316.

Henry, T.B., Menn, F-M., Fleming, J.T., Wilgus, J., Compton, N., Sayler, G.S., 2007. Attributing Effects of Aqueous C60 Nano-Aggregates to Tetrahydrofuran Decomposition Products in Larval Zebrafish by Assessment of Gene Expression. Environmental Health Perspectives 115, 1059-1065.

Henze, M., van Loosdrecht, M.C.M., Ekama, G.A., Brdjanovic, D., 2008. Biological Wastewater Treatment. Principles, Modelling and Design. IWA Publishing, London, UK.

Hou, W.-C., Jafvert, C., 2008. Photochemical Transformation of Aqueous C60 Clusters in Sunlight. Environmental Science and Technology 43, 362–367.

Ko, W.-B., Baek, K.-N., 2002. The Oxidation of Fullerenes (C60, C70) with Various Oxidants under Ultrasonication. Physics of the Solid State 44, 424–426.

230 Lee, J., Cho, M., Fortner, J.D., Hughes, J.B., Kim, J-H., 2009. Transformation of
231 Aggregated C60 in the Aqueous Phase by UV Irradiation. Environmental Science and
232 Technology 43, 4878-4883.
233
234 OECD (1992). OECD Guidelines for the Testing of Chemicals / Section 3:
235 Degradation and Accumulation. Organisation of Economic Co-oporation and
236 Development, Paris, France.
237
238 Oberdörster, E., Zhu, S., Blickley, T.M., McClellan-Green, P., Haasch, M.L., 2006.
239 Ecotoxicology of carbon-based engineered nanoparticles: Effects of fullerene (C60) on
240 aquatic organisms. Carbon 44, 1112–1120.
241
242 Schreiner, K.M., Filley, T.R., Blanchette, R.A., Bowen, B.B., Bolskar, R.D.,
243 Hockaday, W.C., Masiello, C.A., Raebieger, J.W., 2009. White-Rot Basidiomycete-
244 Mediated Decomposition of C60 Fullerol. Environmental Science and Technology 43,
245 3162–3168.
246
247 Standard Methods for the Examination of Waste and Wastewaters (1998) 20th ed.,
248 American Public Health Association/American Water Works Association/Water
249 Environment Federation, Washington DC, USA.
250
251

252 FIGURE LEGENDS

253

254 Figure 1. Development in biological oxygen demand (BOD) expressed in percent of the
255 theoretical oxygen demand tested in OECD 301F tests. I) 10 mgC/L (as NaAc) or 20
256 mgC/L (as nC_{60}); II) 5 mgC/L (as NaAc) and 20 mgC/L (as nC_{60}). After 28 days NaAc
257 (5 mgC/L) was added to flasks containing nC_{60}.

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259 Figure 2. TEM images of nC_{60} suspensions dried on mesh copper grids with holey carbon
260 support films.

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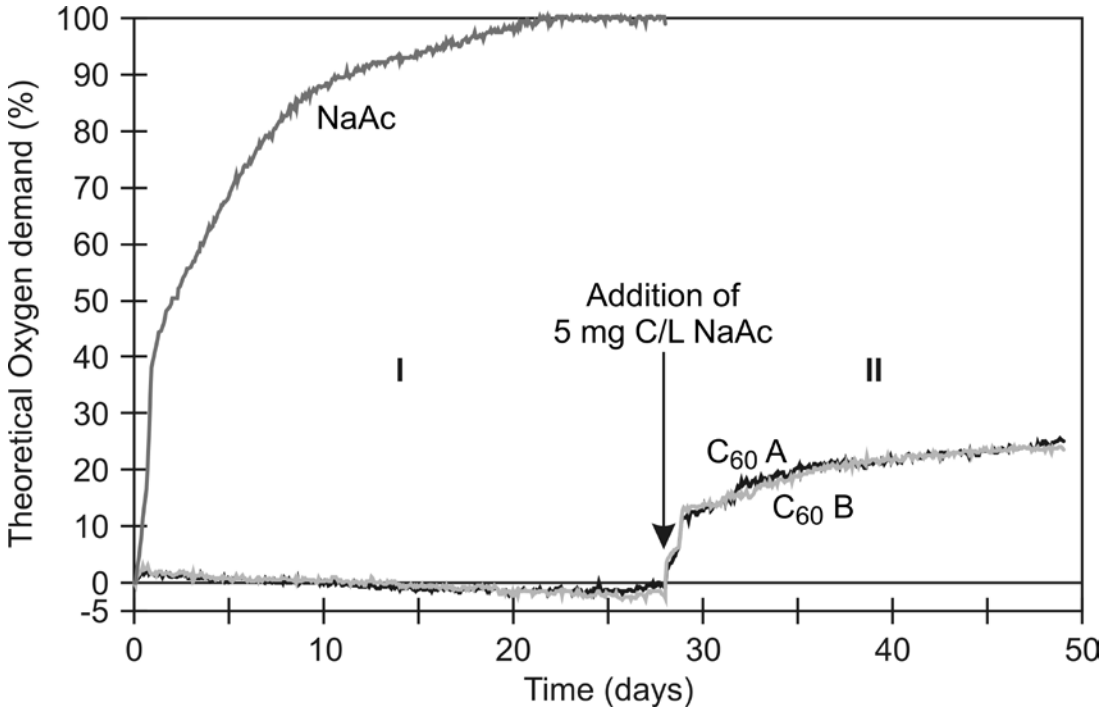
Theoretical Oxygen demand (%)
Time (days)
NaAc
Addition of
5 mg C/L NaAc
I
II
C60 A
C60 B

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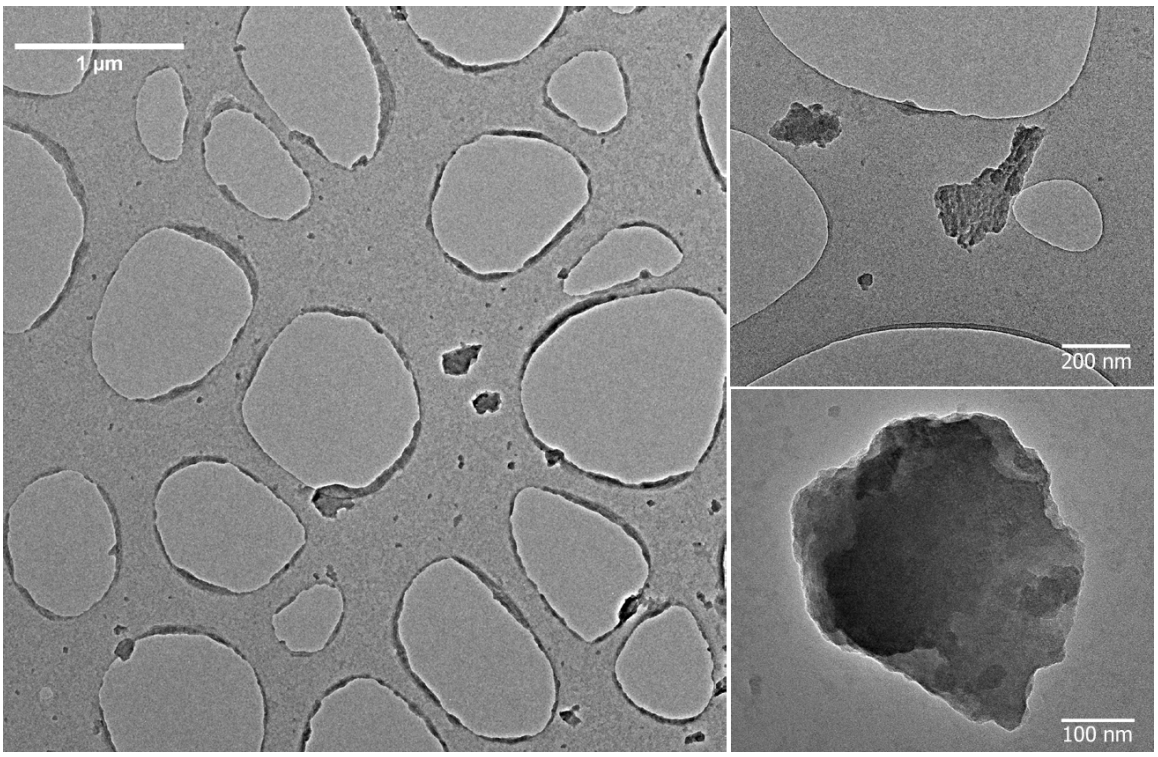
1 µm
200 nm
100 nm

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